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Drugs and the Blood–Testis Barrier

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The functional and morphological evidence for the blood–testis barrier is discussed, together with evidence for the various processes (simple diffusion and facilitated diffusion) by which various substances enter the seminiferous tubule. Data are presented to show that methylmethane-sulfonate (MMS) and dimethylnitrosamine (DMNA) both enter the seminiferous tubules rapidly, although from the published rates of methylation of testicular DNA, by these two compounds, it might be expected that the entry of DMNA would be slower than that of MMS. It appears, however, that DMNA in blood is gradually converted to some nonpermeant compound. The possibility, as yet unsubstantiated, is discussed that a nontoxic permeant precursor may be converted into a nonpermeant toxic substance inside the tubules, thereby effectively concentrating the toxic compound inside the tubules.

The germinal cells in the testis are separated from the blood stream by the blood–testis barrier. Evidence for this barrier can be found in experiments done early in this century involving injections of dyes (*1*), but the significance of these and later observations was overlooked until it was noticed that the exclusion of dye did not occur before puberty (*2*). Physiological evidence for a barrier was forthcoming when techniques were developed for collecting fluid from the rete testis and the seminiferous tubules. Rete testis fluid can be collected by chronic catheterization in several species and acutely in others (*1, 3*). Seminiferous tubular fluid can be collected in rats and hamsters directly by micropuncture (*4–6*) or its composition can be calculated indirectly after efferent duct ligation (*7*). (The ionic composition of tubular fluid calculated by the indirect "difference" technique was found to be very similar to that of tubular fluid collected directly by micropuncture.) Both rete testis and seminiferous tubule fluids are appreciably different in composition from blood plasma or testicular lymph (*3, 4*), and these differences would not be maintained unless there were some restriction on the entry of substances into the seminiferous tubules and rete testis.

By infusing radioactive markers intravenously during the collection of rete testis fluid (*1, 8*) and seminiferous tubular fluid (*6, 7*), it has been shown directly that the exchange of substances between the fluid outside the tubules and the fluid inside can vary over a wide range, from almost instantaneous entry (e.g., tritiated water and ethanol) to almost complete exclusion (e.g. albumin and inulin). The rate of entry does not depend just on molecular size, although in general the larger molecules do enter more slowly; substances with high lipid solubility enter more rapidly than hydrophilic compounds.

The barrier has been located anatomically (*9–11*) at the specialized junctions which develop at puberty between pairs of Sertoli cells (*12, 13*). For some species, the peritubular tissues, particularly the myoid cells, also present some restriction to the entry of large electron-opaque substances (*9, 10*).

Some substances (e.g., glucose) enter the tubules by a process of facilitated diffusion (*14*) and for these compounds there are specific carrier mechanisms operating in the tubular wall, probably in the Sertoli cells. Steroids enter at widely different rates; testosterone enters relatively quickly but the closely related compound 5α-dihydrotestosterone (DHT) enters much less quickly (*15, 16*), although it is slightly more lipid-soluble. Only the free steroid enters the tubules; steroids bound to protein (e.g., after injections of antiserum to an albumin conjugate of the steroid) do not enter the tubules (Fig. 1). This would presumably apply to any drug which is strongly bound to plasma proteins.

Studies with androstenedione are particularly interesting. When radioactive androstenedione was infused intravenously, appreciable amounts of radioactivity appeared in the seminiferous tubule and rete testis fluids. However, practically none of

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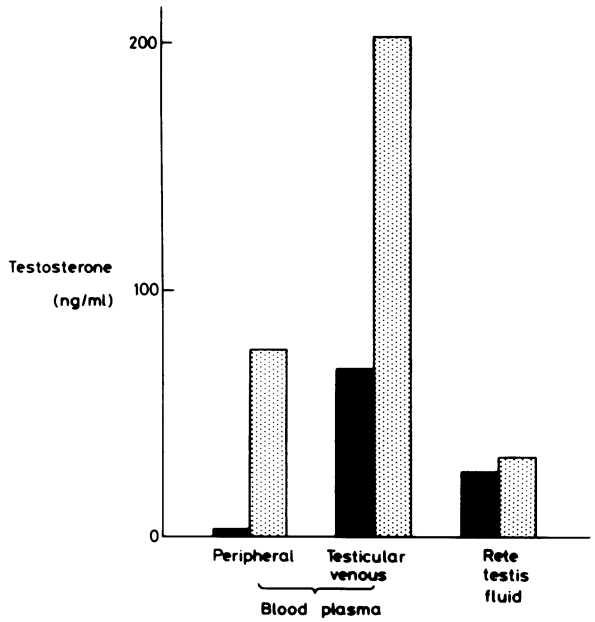

FIGURE 1. Concentrations of testosterone (free + protein-bound) in blood plasma from the aorta and testicular vein and in rete testis fluid: (solid bars) normal rats; (shaded bars) rats injected with a sheep antiserum to testosterone 3-carboxymethyloxime-3-bovine serum albumin (0.6 ml/rat as three intraperitoneal injections over 21 hr; the blood samples were taken 3 hr after the last injection.

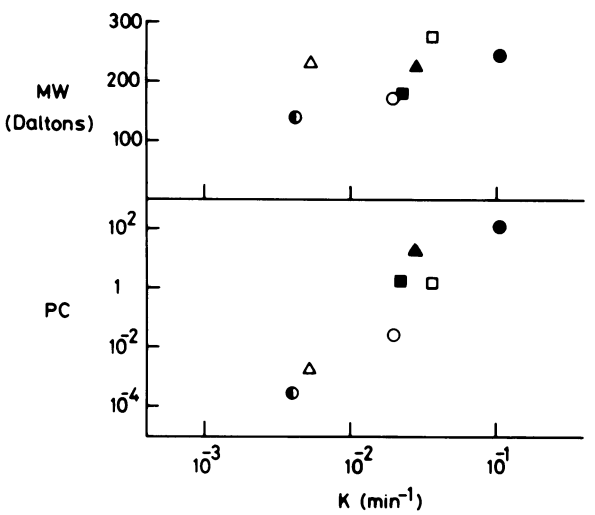

FIGURE 2. Transfer constants, $K = -(1/t) \ln [C_p - (C_{RTF}/C_p)]$ for some barbiturates (closed symbols), and sulfonamides (open symbols), and (○) salicylic acid from blood plasma into rete testes fluid in rats, plotted against the partition coefficient (PC) between chloroform and phosphate buffer (pH 7.4) and molecular weight (MW), where t is time (min) after beginning of infusion; C_P is concentration in blood plasma, and C_{RTF} is concentration in rete testis fluid: (●) thiopental; (▲) pentobarbital; (■) barbital; (○) sulfanilamide; (△) sulfaguanidine; (□) sulfamethoxypyridazine. Note that the high transfer constants are associated with a high chloroform partition coefficient. There is no relation with molecular weights; if anything there is a suggestion that the larger molecules penetrate slightly more quickly. Data from Okamura et al. (*17*).

this radioactivity was present as the infused compound, most being associated with testosterone (*15, 16*). It is likely that this was formed in the interstitial tissue and entered the tubules as testosterone, because a large fraction of the radioactivity in testicular venous blood was also present as testosterone (*16*). These experiments emphasize the need to identify the radioactivity present in the testicular fluids, and, particularly with compounds which may be metabolized by the interstitial tissue, to examine the pattern of radioactivity in testicular venous as well as arterial blood.

Comparatively few studies have been made on the entry of foreign substances into the seminiferous tubules and rete testis. By using a range of barbiturates, sulfonamides, and salicylic acid, it was shown that entry rate depended on lipid solubility, and could vary widely (*17*) (Fig. 2). A number of esters of dimethanesulfonic acid have also been studied; all entered rete testis fluid at appreciable rates (Fig. 3) but had reached only about 20% of blood levels after 2 hr (*18*). The antifertility compound, α-chlorhydrin, enters rete testis fluid almost as quickly as tritiated water (*19*). The entry of two mutagens, methylmethanesulfonate (MMS) and dimethylnitrosamine (DMNA) into rete testis fluid and seminiferous tubular fluid has also been examined. As the methylation of DNA in the testis was

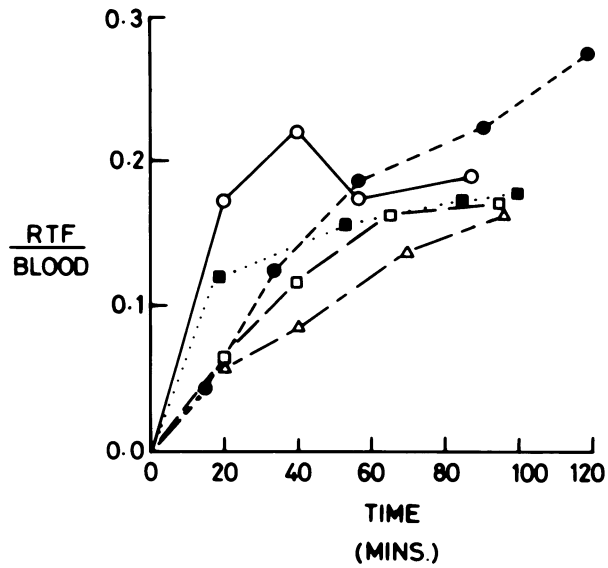

FIGURE 3. Ratios of radioactivity in rete testis fluid to that in blood of rats following intraperitoneal injection of various esters of dimethanesulfonic acid as suspensions in 30% dimethyl sulfoxide–arachis oil (2 ml/kg body weight): (○) methylene dimethanesulfonate (15 mg/kg); (●) ethane-1,2-dimethanesulfonate (2, 50 or 75 mg/kg); (□) propane-1,3-dimethanesulfonate (50 mg/kg); (■) butane-1,4-dimethanesulfonate (Busulphan or Myleran, 4 or 10 mg/kg); (△) nonane-1,9-dimethanesulfonate (9 or 200 mg/kg). The doses used had been previously shown to have antifertility effects. Data from Waites et al. (*18*).

much less with DMNA than with MMS (*20*) it was anticipated that the entry of DMNA would be slower than that of MMS. In fact, total radioactivity had reached equilibrium between rete testis fluid and seminiferous tubular fluid and blood plasma within 1 hr after the injection of either substance (Figs. 4 and 5). It was not possible to identify the nature of the radioactivity present in the various fluids, but it is interesting that the testicular fluids:plasma ratios decreased with time after injection of DMNA. This was presumably due to the appearance of nonpermeant metabolites in the plasma, derived from extratesticular sources.

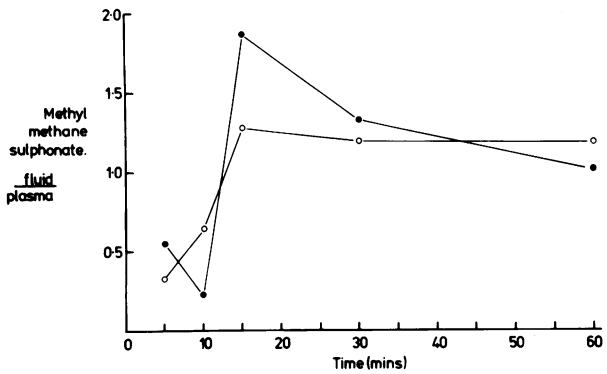

FIGURE 4. Ratios of total radioactivity in (●) rete testis fluid or (○) seminiferous tubule fluid, calculated as described (*7*) for "additional tubular fluid," to that in blood plasma at various times after intravenous injection of [^{14}C] methyl methanesulfonate (Radiochemical Centre, Amersham and Aldrich Chemical Co., 7 μCi, 100 mg/kg body weight).

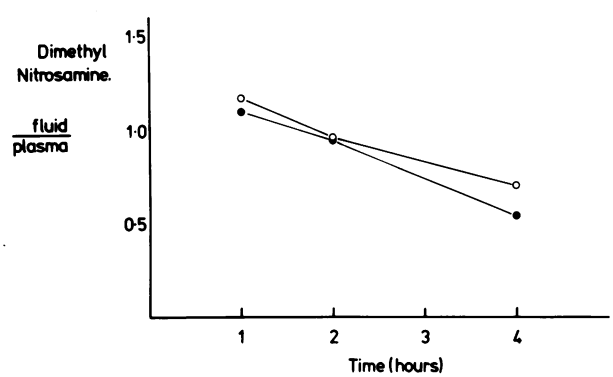

FIGURE 5. Ratios of total radioactivity in (●) rete testis fluid or (○) seminiferous tubule fluid (see Fig. 4) to that in blood plasma at various times after intraperitoneal injection of *N,N*-di[^{14}C] methylnitrosamine (Radiochemical Centre, Amersham and Aldrich Chemical Co., 5 μCi, 30 mg/kg body weight). This dose has no obvious toxic effects on the testes of well-fed rats (*21*).

Another most important theoretical possibility, which has not yet been actually observed, is that a permeant substance could be transformed inside the seminiferous tubules into a nonpermeant, toxic metabolite. Because the latter could not escape from the tubules, it would tend to accumulate there in concentrations much higher than if the toxic metabolite itself were given to the animal. Important mutagenic effects may thereby result at comparatively low doses of the precursor.

Clearly, the blood testis barrier is a very important factor to consider in any studies on drugs which may affect the germinal epithelium of the testis. Lipid-soluble compounds would probably penetrate readily into the tubules but large hydrophilic molecules and substances which bind to plasma proteins usually penetrate only slowly. The barrier could exclude potentially harmful substances from the tubules, but equally it would lead to an accumulation of toxic compounds there.

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